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Mind the gap – context dependency in invasive species impacts: a case study of the ascidian *Ciona robusta*

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Abstract

In the face of increasing invasions and limited resources, appropriate management of invasive species requires prioritisation of species for management action. This process often relies on knowledge of species specific impacts. However, as studies explicitly measuring impact of marine alien species are rare, prioritisation of management actions is often based on studies from outside the geographic area of interest. Further, few impact studies account for context dependency (e.g. seasonal variability or distinct environmental regimes), raising the question of how transferrable knowledge about the impact of a species is between invaded ranges. This study addressed this question by using the widespread invasive solitary ascidian *Ciona robusta* as a case study for assessing impacts across two invaded regions: South Africa and California, USA. We replicated a previously conducted experiment from California that showed that *C. robusta* depresses local species richness in San Francisco Bay. Our South African experiment showed no effect of *C. robusta* on species richness, the Shannon-Weiner diversity index or community composition, despite experiments being carried out over two years and at two depths. While these results may reflect strong density dependency in the impact of *C. robusta*, they serve to highlight context dependency in invasive species impacts. This suggests that until studies of impact in marine systems become common place, context dependency should be explicitly addressed as a source of uncertainty during the prioritisation of species for management action.

Keywords

Ciona robusta, community structure, fouling, impacts, management, species richness

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Introduction

The increasing rate at which alien species are being spread across the globe is well acknowledged (Wonham and Carlton 2005, Ruiz et al. 2011) and resulting invasions are recognised as an important driver of global change (Pysek and Richardson 2010, Simberloff et al. 2013, Blackburn et al. 2014). In response to this, increasing focus has been placed on quantifying impacts associated with invasions (Blackburn et al. 2014) with this body of work strengthening our knowledge about how impacts of invasions manifest at the genetic, individual, population, community and ecosystem level (Parker et al. 1999). Despite general consensus on what constitutes a biological impact by an alien species (i.e. a significant change (increase or decrease) of an ecological property or process, regardless of perceived value to humans (Pysek et al. 2012)), invasion biology still faces the challenge of comparing impacts among invasions (Hulme et al. 2013), a critical step in prioritising appropriate management actions. To this end, recent work has offered a system for classifying alien species based on the magnitude of their ecological impacts (Blackburn et al. 2014, Hawkins et al. 2015). However, this progressive framework remains reliant on the primary studies that document impact, but such studies are surprisingly seldom undertaken for marine alien species (Simberloff et al. 2013, Ojaveer et al. 2015a, Ojaveer et al. 2015b, Alexander et al. 2016). Further, many existing impact studies tend to be unevenly distributed among geographic regions, different taxa and study systems (Pysek and Richardson 2010). Most studies have focused on invasive species (Pysek et al. 2008), leaving the impacts of many other species unquantified, especially in regions where they have not transitioned from alien to invasive status (*sensu* Blackburn et al. 2011). Whilst these gaps in knowledge are understandable as they reflect an uneven distribution of specialist researchers, research funds and research impetus, they hinder appropriate and effective management of invasions (Ojaveer et al. 2015a).

Such management challenges can be particularly relevant in developing nations. For instance, 89 non-indigenous marine species are known from South Africa (Robinson et al. 2016), but impacts have been considered for only 16% of these (Alexander et al. in 2016). Under such circumstances prioritisation of management actions is unavoidably based on studies of impact that have taken place elsewhere, despite a strong call for the application of the precautionary principle under such circumstances (Ojaveer et al. 2015a). This raises the question of how transferrable knowledge about the impact of a species is between invaded ranges.

Due to their prevalence in fouling communities, and the reported ecological or economic impacts of some species, ascidians are often a focal group in marine invasion studies (e.g. Herborg et al. 2009, Rius and Shenkar 2012, Cordell et al. 2013). The *Ciona* complex of solitary ascidians is one such group that has received much attention in the invasion biology literature (see Therriault and Herborg 2008). Recent genetic and morphological studies have, however, recognised that the species nominally referred to as *Ciona intestinalis* in the literature, in fact constitutes two species *Ciona robusta* (also referred to as Ciona Type A) and *C. intestinalis* (also referred to as Ciona

Type B) (Zhan et al. 2010, Sato et al. 2012, Brunetti et al. 2015). Despite a present lack of clarity around the native range of *C. robusta*, it is known from the Mediterranean Sea, the English Channel, Japan, the west coast of north America, the south east coast of Australia, as well as from South Africa and New Zealand and is thought to be native in the Mediterranean Sea and the English Channel (Zhan et al. 2010, Rius et al. 2016). Due to its limited larval dispersal capabilities and the sessile nature of its adult phase, *C. robusta* (referred to as *C. intestinalis*) is thought to have been translocated primarily via hull fouling (Lambert and Lambert 2003) and aquaculture transfers (Castilla et al. 2005), with intra-regional spread closely associated with recreational boating (McDonald 2004). Despite its large introduced range, the ecological impacts of *C. robusta* have only been considered in San Francisco Bay, California, where it was found to reduce diversity of sessile communities (Blum et al. 2007). Although economic impacts on shellfish farms are widely reported for *C. intestinalis* (Lesser at el. 1992, Tan et al. 2002, Carver et al. 2003, Braithwaite and McEvoy 2004), such impacts by *C. robusta* have received less attention (but see Robinson et al. 2005, Rius et al. 2011).

Previously referred to as *C. intestinalis*, *C. robusta* has been known from South Africa for more than 50 years (Millar 1955), where it occurs extensively in harbours along almost the entire coastline (Rius et al. 2014). Despite this there has been no consideration of its ecological consequences. As such, an opportunity exists to use this species as a case study to consider the transferability of information on ecological impacts of alien species between two different invaded ranges, in this case between the west coast of North America and South Africa. We did this by repeating the experiments of Blum et al. (2007) so as to gain a measure of ecological impact by *C. robusta* in South African waters. Specifically, we assessed the effect of this ascidian on sessile community structure and species richness at two depths, over two years. This enabled an assessment of the spatial and temporal variability associated with the impacts of *C. robusta* across distinct biogeographical regions.

Methods

This study took place at two locations, Yacht Port Marina (33°01'36"S; 17°57'40"E) in Saldanha Bay on the South African west coast and Gordons Bay Yacht Club (34°09'52"S; 18°51'42"E) in False Bay on the south coast (Figure 1). These sites were chosen as they are both sheltered from wave action and currents by breakwaters that protect moored yachts from rough sea conditions. A pilot study that included sites with greater water movement revealed that *Ciona robusta* preferentially settled in sheltered conditions and thus more exposed sites were excluded. While these are both enclosed yacht basins, they differ in that they occur in distinct ecoregions, Saldanha Bay in the cool nutrient rich Southern Benguela Ecoregion and False Bay in the warmer less productive Agulhas Ecoregion (Sink et al. 2012).

During the austral winter of 2012 and 2014, 18 experimental arrays were deployed in Saldanha Bay and False Bay. This season was chosen as this is when the peak settle-

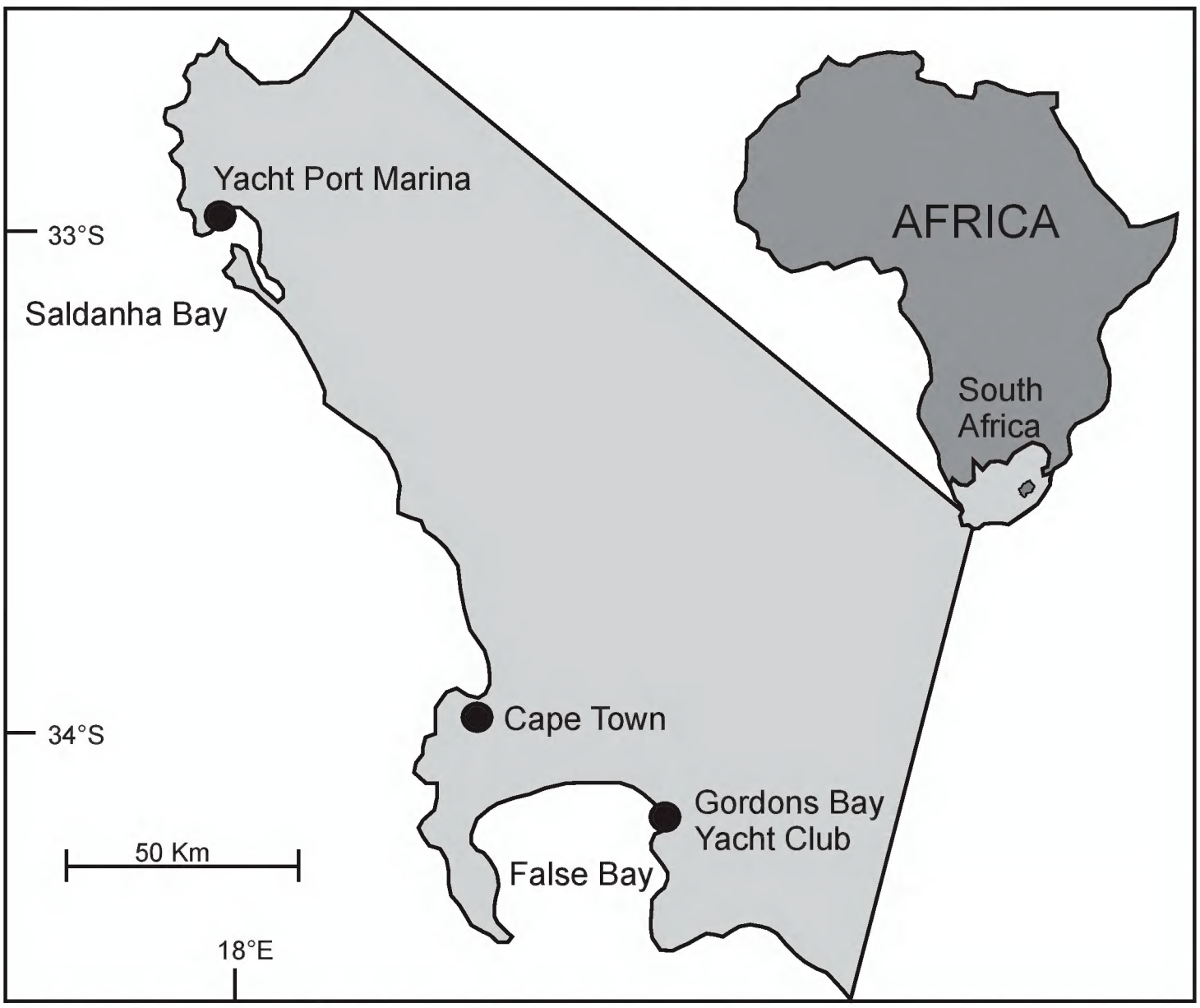

Figure 1. Sites along the South African coast where the ecological impacts of *Ciona robusta* were quantified.

ment of *C. robusta* occurs in this region (Millar 1955). Despite adult *C. robusta* being present in False Bay in pre-experiment surveys, no settlement occurred in either experimental year forcing this site to be excluded from all statistical analyses. Each array hung vertically in the water column and consisted of two PVC panels, one at 1m depth (i.e. shallow plates) and another at 3m (i.e. deep plates). Panels were opaque in colour, 0.25 cm thick and offered a settlement area of 20 × 20 cm. This size was chosen to align with the 'large' panels used by Blum et al. (2007). Prior to deployment the sanded plates were soaked in sea water for two weeks in order to leach chemicals that may have affected fouling. The arrays were randomly allocated to one of three treatments: (1) *Ciona* removal (n=6), where all *C. robusta* were removed from the plates by hand at two weekly intervals; (2) the treatment control (n=6), where plates were removed from the water for the same length of time as the treatment plates but without removing *C. robusta* to control for treatment artefacts; and (3) the control (n=6), where plates were left undisturbed for the duration of the study. Thus, each array had a shallow and deep plate, providing n=6 for each treatment at each depth. This sample size allowed for 0.91 power to detect the effect size recorded by Blum et al (2007), which is above the level of 0.8 advocated by Cohen (1977). The individuals removed from the *Ciona* removal treatment were counted, and

wet weighed to the nearest gram and compared among years (2 levels: 2012 and 2014) and depths (2 levels: shallow and deep) in a GLM, with quasipoisson error distribution to account for overdispersion. Plates were deployed for 16 weeks after which they were removed from the water, photographed and preserved in Formalin. Back in the laboratory, percentage cover was measured using 25-point counts generated by the random placement of a 5 by 5 grid on the photographs taken in the field. In addition, biota from each plate were identified to species level and weighed to the nearest 0.1 gram.

Species richness (i.e., total number of species) and the Shannon-Wiener diversity index (H'), which incorporates both species richness and evenness (Clarke and Warwick 1994) were used to compare diversity among treatments (3 levels; control, treatment control, Ciona removal), years (2 levels: 2012 and 2014) and depths (2 levels: shallow and deep) in a three-factor GLM, with quasipoisson error distribution. Calculations of H' were based on biomass as many fouling species are colonial, precluding counts of individuals. Using the Primer-6 software package (version 6.1.16) a PERMANOVA (version 1.0.6) was used to assess differences between fouling community assemblages among treatments, years and depths (Anderson et al. 2008). Multi-dimensional scaling (MDS) plots were used to visualise the relationships between communities while SIMPER was used to isolate the species responsible for differences in community structure. All multivariate analyses were conducted using non-standardized, fourth-root transformed biomass and cover data. The contribution made by *C. robusta* was excluded from these analyses so as to isolate its effect on the fouling community and not simply reflect its absence from treatment plates. All univariate statistics were performed in R version 3.2.0

Results

Settlement of *Ciona robusta* on experimental plates

Ciona robusta only settled on experimental plates in Saldanha Bay, despite pre-experiment surveys recording this ascidian in Gordons Bay and the presence of low densities of adults on marina infrastructure during the experiment. As such Gordons Bay was excluded from all analyses. In Saldanha Bay, both the number and biomass of individuals removed from the treatment plates were affected by 'depth' (density: $F_{1,21}$=24.32, p<0.0001, biomass: $F_{1,21}$=24.16, p<0.0001) with significantly lower abundances occurring on shallow plates (density: t=-2.16, p<0.05; biomass: t=-1.71, p<0.05) (Figure 2). There was no effect of 'year' (density: $F_{1,22}$=0.53, p>0.05, biomass: $F_{1,22}$=1.08, p>0.05) and no interaction between 'depth' and 'year'.

Impacts on diversity and community composition

In total, 58 fouling species were recorded in our study, of which 57% were only present in 2012. While a total of seven non-indigenous species were recorded, only *C. robusta*,

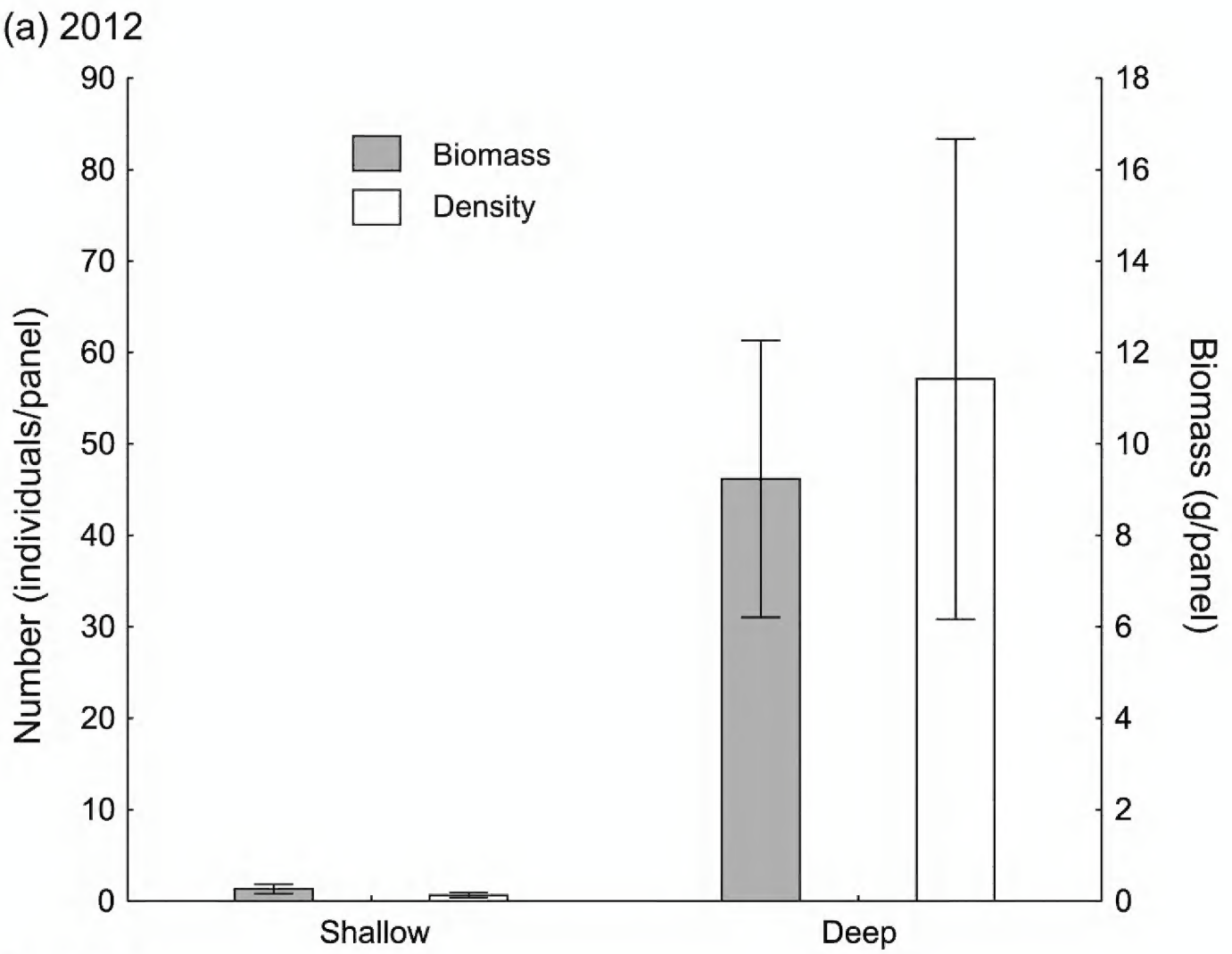

Figure 2. Abundance of *Ciona robusta* removed from treatment plates. Mean (±SE) numbers (individuals/panel) and biomass (g/panel) of *C. robusta* removed from plates in Saldanha Bay in (**a**) 2012 and (**b**) 2014.

Table 1. GLM results considering the effect of 'year', 'depth' and 'treatment' on (a) species richness and (b) the Shannon-Wiener diversity index (H'). ns = non-significant.

Factor	df Effect	Null deviance	F-ratio	*p*-value
(a) Species richness				
Year	1	142.6	103.4	$p<0.001$
Treatment	2	3.1	1.1	ns
Depth	1	6.5	3.2	ns
Year × Treatment	2	3.1	1.1	ns
Year × Depth	1	12.8	9.3	$p<0.01$
Treatment × Depth	2	0.4	0.2	ns
Year × Treatment × Depth	2	2.6	0.9	ns
(b) H'				
Year	1	5.3	71.5	$p<0.001$
Treatment	2	0.2	1.0	ns
Depth	1	1.0	2.2	ns
Year × Treatment	2	0.2	1.2	ns
Year × Depth	1	1.2	15.4	$p<0.001$
Treatment × Depth	2	0.009	0.1	ns
Year × Treatment × Depth	2	0.2	1.3	ns

the colonial bryozoan *Bugula neritina* and the lightbulb ascidian *Clavelina lepadiformis* were present in both years, while the remaining four species (the ascidian *Diplosoma listerianum*, the amphipod *Jassa marmorata*, the hydrozoan *Obelia dichotoma* and the bryozoan *Waterspora suborquata*) were present only in 2014, despite fewer species being recorded in that year. Only one species, *C. lepadiformis,* was restricted to removal treatment plates. There was a significant effect of 'year' on species richness and a significant interaction between 'year' and 'depth' (Table 1). Overall species richness was highest in 2012 (t=-3.88, $p<0.01$) with elevated richness on deep plates in 2014 driving the interaction (Figure 3a, c). There was no effect of 'treatment' on species richness. The same pattern emerged for the Shannon-Wiener diversity index (Table 1, Figure 3b, d).

Community assemblages differed significantly among years and were affected by an interaction between 'year' and 'depth' (Table 2, Figure 4). Treatment was found to have no effect on community structure. These patterns were observed for community structure based on biomass and % cover. SIMPER on fouling biomass revealed that the 2012 community was defined primarily by *O. dichotoma,* the indigenous barnacle *Notomegabalanus algicola,* and the ascidian *Botryllus magnicoecus*. Together these species contributed 40.1% to the similarity of communities recorded in this year. In contrast, 2014 communities were primarily designated by *B. neritina* (contributing 32.3%) followed by *B. magnicoecus* (12.4%) and *Botryllus schlosseri* (12.3%). A SIMPER analysis on % cover of biota again highlighted the importance of colonial ascidians in fouling communities, with *B. magnicoecus* and *Diplosoma listerianum* contributing 42.3% to the similarity of 2012 communities, while *B. schlosseri* and *B. magnicoecus* accounted for 36.1% of similarity in 2014 assemblages.

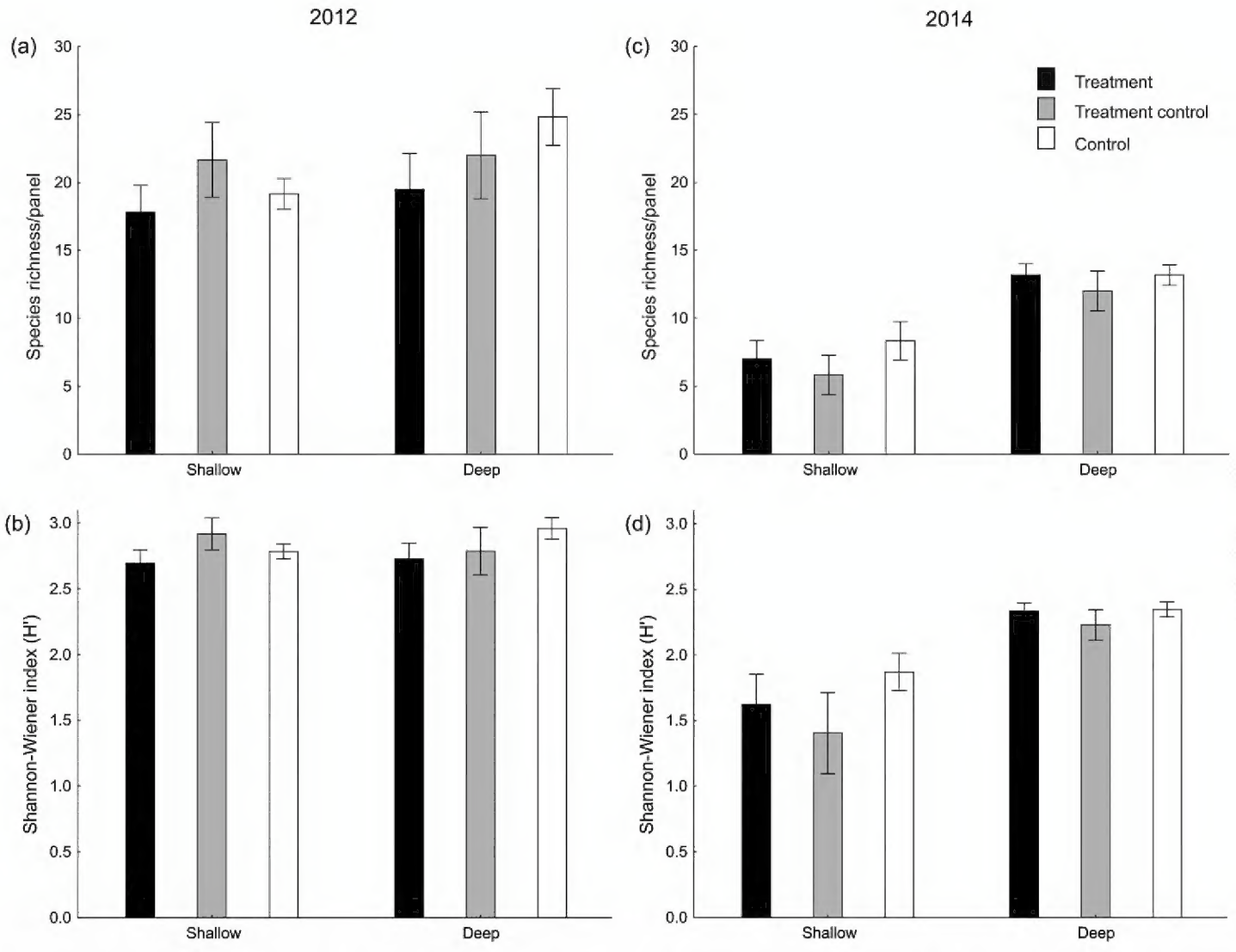

Figure 3. Changes in diversity. Mean (±SE) species richness and Shannon-Wiener index (H') recorded in 2012 (**a, b**) and 2014 (**c, d**). Both measures of diversity were significantly affected by year ($p<0.01$) and a significant interaction between year and depth ($p<0.01$).

Table 2. Test statistics for a main effects PERMANOVA considering the effect of year, treatment and depth on fouling (a) biomass and (b) % cover. ns = non-significant.

Factor	df	SS	MS	Psuedo-F	*p*-value
(a) Biomass					
Year	1	60124	60214	57.8	$p<0.0001$
Treatment	2	32785	1393	1.1	ns
Depth	1	23966	23966	4.8	ns
Year × Treatment	2	2506	1253	1.2	ns
Year × Depth	1	5032	5032	4.8	$p<0.001$
Treatment × Depth	2	2493	1246	1.9	ns
Year × Treatment × Depth	2	1291	646	0.6	ns
(b) % cover					
Year	1	49301	38454	65.3	$p<0.01$
Treatment	2	36247	1668	1.6	ns
Depth	1	26354	26354	3.2	ns
Year × Treatment	2	3803	1969	1.8	ns
Year × Depth	1	6712	5644	9.4	$p<0.05$
Treatment × Depth	2	2113	1746	2.1	ns
Year × Treatment × Depth	2	1394	452	0.3	ns

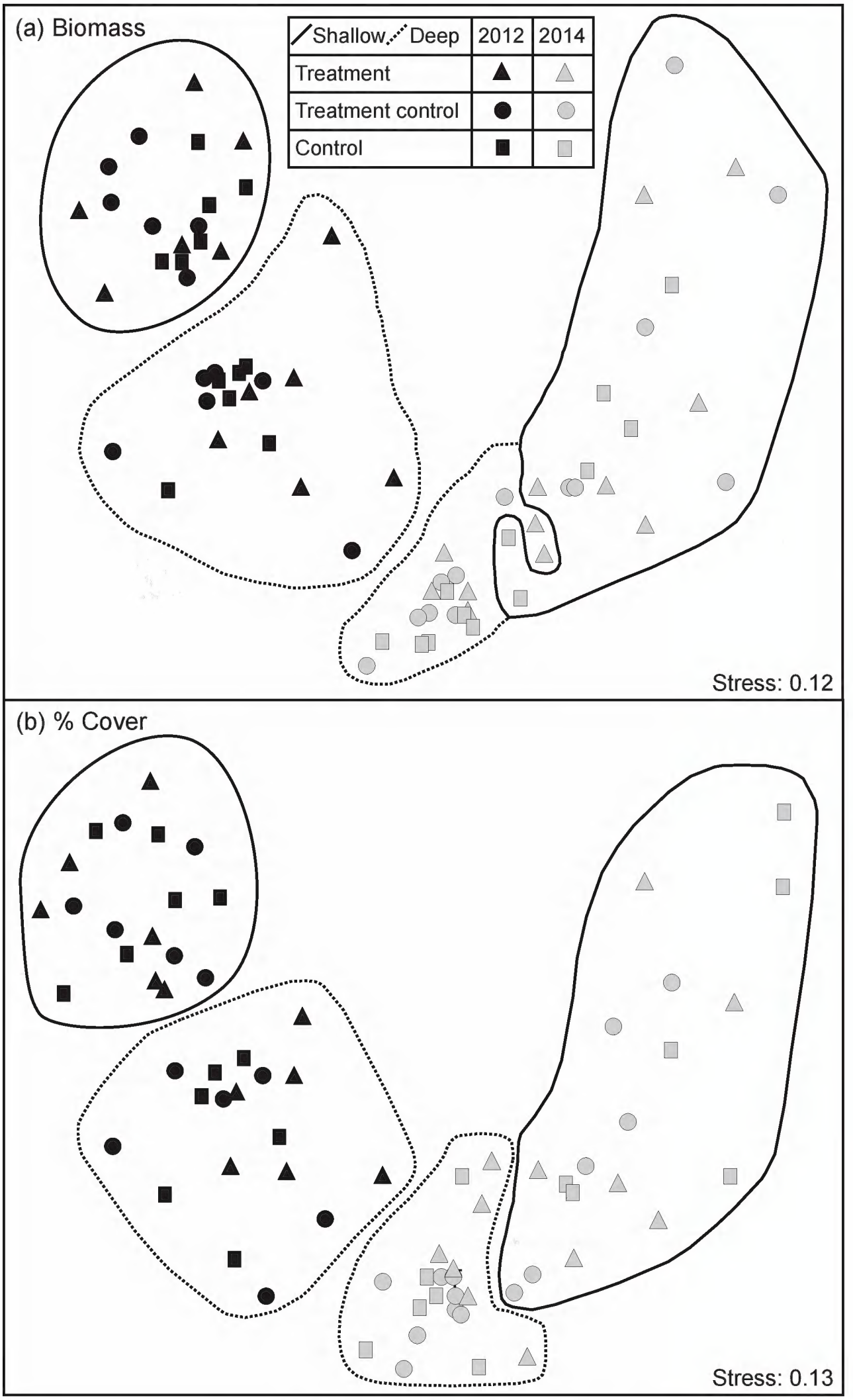

Figure 4. Community composition. Multi-dimensional scaling (MDS) plots of community assemblages formulated using fouling (**a**) biomass and (**b**) % cover of treatment, treatment control and control panels in two years at two depths.

Discussion

Alien species can have ecological, socio-economic and human health impacts in recipient regions (Mack et al. 2000, Kumschick et al. 2012, Simberloff et al. 2013). Faced with escalating rates of invasions and limited resources, managers are required to prioritise responses to species incursions (Kumschick et al. 2012), with a recent trend to do so based on the relative impacts associated with the species in question (Blackburn et al. 2014). Due to the paucity of studies directly measuring the impact of marine alien species (Ojaveer et al. 2015a), prioritisation often relies on the published literature as: (1) managers must strive for proactive and efficient management actions, an approach that inherently minimises the time available to measure impacts, and/or (2) limited resources preclude experimental assessments. Using the solitary ascidian *Ciona robusta* as a case study, we have shown differential impacts between distinct global locations where this species has been introduced. In California, *C. robusta* was shown to reduce local species richness and alter sessile community structure, whereas our work in South Africa found no ecological impacts of this species on the local fouling community.

Although previous studies have recorded dense settlement of *C. robusta* in Saldanha Bay (in 1994 an average density of more than 1000 individuals/m^2 was recorded at a depth of 3m (Rius et al. 2011)), it appears that densities may be declining through time in this area as fewer than 500 individuals/m^2 were recorded in 2010 (Rius et al. 2011) and we noted average settlement of less than 100 individuals/m^2 in 2012 and fewer than 50 individuals/m^2 in 2014. Further, when compared to much relatively higher densities reported by Blum et al. (2007), this suggests that the impacts of *C. robusta* may be density dependant, as has been recorded for other alien species (Griffen and Byers 2009). This aligns with the suggestion by Thomsen et al. (2011) that invasion impacts depend primarily on the properties associated with the alien species itself (e.g. density or species identity) and secondarily on the characteristics of native biota (e.g. indigenous community structure), resource levels (e.g. nutrient levels) and abiotic conditions (e.g. sedimentation), but remains to be empirically tested. What remains unclear, however, is what may be driving the decline of this ascidian which has been present along this coast for more than half a century (Millar 1955) and is reported from almost all South African harbours (Peters et al. 2014, Rius et al. 2014). One possible explanation relates to long-term cooling trends in water temperature along the South African west coast (Rouault et al 2010) as recruitment of this species may be closely correlated with this environmental variable as it is for *Ciona intestinalis* (Vercaemer et al. 2011). However, a decadal decline of 0.5°C and the fact that temperatures in Saldanha Bay (Smit et al. 2013) fall within the thermal range of other locations at which this species persists (Zhan et al. 2010), suggests that temperature is not the sole driver of declines in recruitment. This does, however, raise questions about recruitment trends of this ascidian along warmer sections of the South African coast and the potential impact in these regions.

Conclusion

While the impacts of alien species are often measured at different locations within a region (e.g. Dunham et al. 2002) or in different invaded ranges (e.g. Kado 2003; Sadchatheeswaran et al. 2015) and then assimilated in reviews and meta-analyses (e.g. mice on islands (Angel et al. 2009), global plant impacts (Vila et al. 2011), biological impacts of ascidians (Aldred and Clare 2014)), studies directly comparing impacts at the species level in different invaded ranges are generally conspicuous by their absence. While the theoretical framework for understanding variability in the manifestation of impacts is developing (Thomsen et al. 2011, Ricciardi et al. 2013), empirical studies are needed to support this (Thomsen et al. 2011). Although identifying potentially high risk species based on impacts reported from elsewhere remains useful, and impact quantification is obviously not practicable for every alien species in every invaded range, engaging in expensive management actions without ground truthing the applicability of reported impacts to the area of interest is also not prudent or efficient. In acknowledging the need for more quantitative studies considering the impacts of marine alien species (Wardle et al. 2011, Alexander et al. 2016) recent work has suggested that impact evaluation for data deficient marine systems should focus on the value sets that management actions seek to protect (Ojaveer et al. 2015a). While this precautionary approach aims to support management in the interim, there is a dire need for quantification of impacts to support evidence based management and provide data with which to test and develop our conceptual understanding of context dependency in invasion biology.

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